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Secretion of Insulin During Aging

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Recognition of and response to environmental challenge are expressed in part by the availability and efficacy of circulating hormones for the modulation of intermediary metabolism in target cells. One example of such adaptive capability that changes during aging is the impact of dietary glucose on its insulin-sensitive metabolism by liver and peripheral tissues. This paper provides a critical review of published research that addresses the pivotal role of insulin secretion in that adaptive sequence during aging.

According to most, but not all, published reports, the regulation of insulin secretion by glucose changes during aging. However, interpretation of these data is complicated by numerous factors, most prominent of which include 1) the extent to which it is possible to evaluate insulin secretion in vivo by measurement of hormone levels in peripheral blood; 2) the distribution of functionally heterogeneous populations of pancreatic islets of Langerhans; 3) distinctions between growth, obesity, and aging; and 4) the frequent failure to acknowledge relevant, previously published literature. The present paper will examine these data both in the context of such complications and from the perspective of the pursuit of further insight into the fundamental biological processes of aging that are expressed in the absence of disease and inappropriate lifestyle.

ALTERED RESPONSIVENESS TO GLUCOSE IN VIVO

When the pancreas recognizes and responds to an increased level of blood glucose, insulin is secreted into the portal vein and through it is transported immediately to the liver. Within the liver insulin exerts certain of its hormonal actions and also is metabolized. The proportion of insulin molecules that escapes its hepatic metabolism is distributed throughout the peripheral circulation and tissues. Therefore, assessment of insulin secretion by the pancreas into the circulation can be approximated most accurately in portal vein blood.

The concentration of insulin in portal vein blood is the net result of its secretion into the blood by the pancreas and its extraction from the blood by the liver at early points during the secretory response, although recirculation of secreted insulin complicates matters increasingly with the passage of time. However, the concentration of insulin in peripheral blood at any given time represents the far more complex net result of its pancreatic secretion, hepatic clearance, uptake and clearance through peripheral tissues, recirculation, and disposition within the blood. Therefore, consideration of peripheral blood insulin levels during aging in the context of the regulation of insulin secretion is excluded from this review, although previous work by Chen et al,[1] for example, is particularly relevant in this regard.

The pattern of insulin response to glucose detected in portal vein blood of fasted rats during aging is complex. Gold et al[2] injected an aqueous solution of 450 mg glucose per 100 g of body weight intragastrically into three-day fasted, male Sprague-Dawley rats ranging in age from two to 24 months. At two months of age the initial phase of a biphasic response to glucose is detectable almost instantaneously and is transient. At 12 and 24 months of age the initial phase of insulin response increases in magnitude and duration relative to that observed at two months. The second phase of response begins approximately 30 minutes subsequent to the glucose challenge and endures for several hours at two months of age. However, its time of onset is progressively delayed from 30 minutes following glucose challenge at two months of age, to three to four hours at 12 months, and to nearly seven hours at 24 months. Under these experimental conditions, aortal blood glucose concentration peaks within several minutes at a level of approximately 220 mg/100 mL and maintains a plateau level of approximately 160 mg/100 mL for at least seven hours at all ages tested.

Interpretation of these data within the context of se-

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cretory capability is difficult. For example, the hyperinsulinemia associated with the early phase of response by older, fasted Sprague-Dawley rats may be a consequence of obesity. Why such hyperinsulinemia is not also expressed during the second phase of response is unclear. Possible differences in responsiveness to glucose in the fed and fasted states were not explored. Furthermore, apparently reduced rates of insulin clearance during aging[3-7] may also contribute to the altered pattern of response. There apparently are no published data on portal vein insulin response in vivo to stimuli other than glucose during aging.

Efficacy of the insulin molecules secreted into portal vein blood in response to administered glucose may diminish indirectly during aging as a consequence of the enhanced presence of glucagon, the primary physiological antagonist of insulin action on the liver. The suppression of glucagon secretion into portal vein blood by glucose challenge during fasting is progressively inhibited as Sprague-Dawley rats aged from two to at least 24 months.[8] Circulating levels of glucose in this experiment were identical to those utilized previously[2] to generate the portal vein insulin response; approximately 160 mg/100 mL. Similar observations also were reported in aging Wistar rats,[9,10] although interpretation of these latter measurements is complicated by the assessment of glucagon concentration in peripheral blood, as discussed above.

The molar ratio of glucagon to insulin in portal vein blood may be sufficiently high during the initial hours of response to glucose by older fasted rats that the sluggish adaptation by hepatic glucokinase observed previously[11] is possible only at delayed times of onset as excess glucagon levels diminish. Inhibition of the glucokinase adaptation by simultaneous treatment of two-month-old, fasted rats with glucose and glucagon[8] supports such a notion. Whether or not the simultaneous treatment of aging rats with glucose and either antibodies to glucagon or some pharmacological glucagon-blocking agent is capable of overcoming the sluggishness of the glucokinase adaptation remains to be seen. Furthermore, interactions between glucagon and liver during aging were not investigated under these experimental conditions.

Whether or not intrinsic biological potency of insulin molecules secreted in vivo changes during aging is uncertain. Proinsulin-like material is reported to accumulate in peripheral blood of aging people.[12] However, as already discussed above, it is difficult to interpret peripheral measurements of insulin in the context of insulin production.

ALTERED RESPONSIVENESS TO GLUCOSE IN VITRO

The altered insulin response to glucose in vivo detected in portal vein blood of aging, fasted Sprague-Dawley rats[2] probably reflects, at least in part, a change in the pancreatic regulation of the glucose-stimulated secretion of insulin. However, the specific nature of the insulin response in vitro, including those reports that profess the absence of any impact of aging, is highly dependent on the protocol utilized for the in vitro secretory system, as well as various physiological characteristics of the donor animal. Of those numerous factors that influence the observed patterns of insulin secretory response in vitro at different donor ages, the most noteworthy include physical size of the islets tested and response time at which insulin levels are measured. Magnitude and time course of insulin response to glucose by islets of heterogeneous sizes differ considerably at given donor ages.[13] Furthermore, the distribution of islet sizes changes as donors age.[14-18] Consequently, much of the published data on glucose-stimulated secretion of insulin in vitro may require reevaluation.

The pattern of glucose-stimulated insulin response detected in vivo by Gold et al[2] in portal vein blood of fasted, male Sprague-Dawley rats aged two to 24 months is expressed in part in vitro when insulin secretion is stimulated by perifusion of isolated islets of different sizes from corresponding donor rats, as described by Kitahara and Adelman.[13] The insulin secretory response to 16.7 mmol (300 mg/100 mL) glucose by perifused small (50-80 μm diameter) islets from fasted two-month-old rats is delayed by approximately two hours when assessing the identical islet population from fasted 24-month-old rats. This delayed time of onset resembles that observed in vivo for the second phase of insulin response in portal vein blood.[2] In contrast, the insulin secretory response by perifused large (350-400 μm diameter) islets from fasted two-month-old rats is several times greater than that of small islets, and is only very slightly inhibited, but not altered in time course, as donor rats age from two to 24 months.

The greater prevalence of large islets in the pancreas of older rats[14-18] may account for the continued ability of the pancreas in fasted, 24-month-old rats to secrete insulin in vivo into portal vein blood rapidly in response to a glucose challenge, a capability that deteriorates in small islets in vitro as donor rats age. Perhaps the larger islets also contribute to the hyperinsulinemia of first-phase insulin response to glucose in vivo at 12 and 24 months of age. The change in distribution of heterogeneous islet populations during aging is not understood. Whether or not the greater prevalence of large islets in the pancreas of aging rats represents compensatory islet hypertrophy in response to the impaired capability of smaller islets for glucose-stimulated secretion of insulin, as already speculated,[13,19] awaits experimental evidence.

Reaven et al[19] reported that the magnitude of a glucose-stimulated (25 mmol; 450 mg/100 mL) insulin response by an islet incubation system diminishes progressively as donor Sprague-Dawley rats age from two to 18 months, whereas the number of islet beta cells and

islet insulin content increases. This study selected islets from a size (diameter) range of 200–400 μm, and the islet population examined from progressively older rat donors contains progressively larger islets than does the islet population examined from younger rat donors. Furthermore, insulin response was measured only at the 60-minute time-point following incubation of islets with the stimulatory concentration of glucose.

The apparent reduction of insulin responsiveness during aging under these conditions is probably the consequence of a complex change in distribution of functionally distinct islet populations. The secretory contribution by the population of small (50–80 μm diameter) islets described by Kitahara and Adelman,[13] which has a time of onset delayed beyond the utilized 60-minute incubation period during aging, is apparently not included in this study. Whether or not the secretory contribution by some proportion of the smaller of those islets selected by Reaven et al[19] is progressively diminished in a similar fashion as donor rats age is uncertain. The secretory contribution by larger islets, prevalence of which increases during aging, is progressively enhanced during aging. Therefore, the number of islet beta cells and islet insulin content are increasing in an islet population that is distinctly different than the population that is losing insulin responsiveness most prominently (the smaller islets) as donor rats grow older. The identical issues surrounding heterogeneity of islet size also obfuscate interpretation of another report by Reaven et al[20] that pancreatic regional differences cannot explain the reduction in glucose-stimulated insulin response in vitro by incubated islets as donor Sprague–Dawley rats age from two to only 12 months.

Exercise and caloric restriction are equally effective in preventing the age-related increase in islet volume, whereas exercise seems to be more effective in preventing the age-related decrease in insulin secretion by isolated islets from Sprague–Dawley rats, according to Reaven and Reaven.[21] However, once again it is necessary to reinterpret the data in a context that more appropriately reflects their utilized protocol for selection of islet population. Exercise and caloric restriction reduce the contribution by larger islets to the heterogeneous size distribution (200–400 μm diameter) of islets otherwise observed in this study as Sprague–Dawley rats age from two to 12 months. In fact, tabulated volumes of islets that actually were utilized for insulin secretion studies are reported to differ significantly only when comparing two- and 12-month-old controls. As already discussed above, the apparent reduction of insulin responsiveness under these conditions is probably the consequence of a complex change in distribution of functionally distinct islet populations.[13]

At the same time, observed rates of glucose-stimulated insulin secretion by incubated islets from two-month-old control, 12-month-old exercised, and 12-month-old calorie-restricted rats were probably obtained using islet populations of similar, near homogeneity.[21] Therefore, it seems appropriate to recalculate their data on the basis of the number of islets incubated, rather than normalized per islet volume, as published. When this is done, the mean rates of insulin secretion in response to the highest concentration of glucose used are as follows: two-month control (1.79 μU insulin/min per 20 islets); 12-month exercised (1.65 μU insulin/min per 20 islets); and 12-month calorie-restricted (1.34 μU insulin/min per 20 islets). According to their statistical analysis, rate of insulin secretion by islets from calorie-restricted, but not exercised, 12-month-old rats differ significantly from that of two-month-old controls at the highest glucose concentration. This probably means that exercise is more effective than caloric restriction in preventing a modest decrease in the glucose-stimulated insulin secretion that characterizes large islets as donor rats age from two to only 12 months—perhaps by minimizing the obesity that otherwise characterizes the Sprague–Dawley rat in this early portion of its lifespan. Any impact of exercise or calorie-restriction on the much greater loss of capacity for glucose-stimulated insulin secretion by small islets from 24-month-old donor rats[13] must await further experimentation of this kind on the appropriate islet population from old rats.

Leiter et al[22] reported that glucose-stimulated insulin secretion by perifused islets in vitro is not impaired as male C57B1/6J mice age from five to 25 months. In particular, the authors indicated that too little prior effort had addressed age-related changes in glucose homeostasis in specific-pathogen-free (SPF) colonies of inbred mice without recognized diabetes or obesity genes, such as the C57B1/6J mouse. Consistent with their observation is a report by De Clerq et al[23] that the cumulative amount of insulin secreted into the media by long-term culture of islets in glucose, as well as their subsequent response to glucose-stimulation in an incubation system, does not diminish as donor age of Wistar rats increases from three to 30 months. However, relative islet size selected at each donor age before establishment of cultures is not indicated.

Interpretation of the data by Leiter et al[22] may be complicated by the choice of islet populations for at least the following three reasons: First and foremost, the secretory contribution by the population of small (50–80 μm diameter) islets described by Kitahara and Adelman,[13] time of onset for which is delayed as donor Sprague–Dawley rats age from two to 24 months, is apparently not included in the study by Leiter et al.[22] Furthermore, as indicated in their protocol, medium-sized islets isolated and utilized from 25-month-old mice were at least 1.5 times larger than those from the pancreases of five-month-old mice. Whether or not the secretory contribution by some proportion of the smaller of those medium-sized islets selected by Leiter et al[22] is progressively impaired in a similar fashion as donor rats age is uncertain. Second, the secretory contri-

bution by larger islets, prevalence of which increases during aging, is progressively enhanced during aging. Finally, the increase in islet size observed in this animal model during aging is presumably not the consequence of obesity. It is tempting to speculate that larger islets do not lose insulin responsiveness to glucose in vitro, as the donor ages, when the donor is not obese. Such a likelihood is consistent with the observation by Reaven and Reaven[21] that the exercise-induced lessening of obesity in Sprague-Dawley rats aged two to 12 months minimizes the loss of insulin responsiveness in vitro by larger islets from older donors, as discussed above.

Criticism by Burch et al[24] of earlier research on impairment of insulin responsiveness to glucose during aging by Reaven et al[19,25] suggests that apparent deficits may be an artifact caused by the more aggressive digestion needed to free islets from the tougher connective tissue of older animals. The former group[24] appropriately demonstrated the critical importance of the technique utilized for islet isolation to the observed pattern of stimulated insulin release. However, their criticism of earlier observations on impaired stimulation of insulin release during aging is not appropriate for at least the following three reasons: 1) Their selection of islet population for perifusion incorrectly masks age differences in glucose-stimulated insulin response. In average-sized islets, age differences in insulin response are minimized because insulin release is impaired to a greater extent in smaller islets.[13] 2) Age differences in glucose-stimulated insulin response are minimized unnecessarily by the selection of 10 and 14 months as old Wistar rats, and 18 months as old Sprague-Dawley rats.[26] 3) The authors fail to acknowledge previous literature that documents the impact of aging on glucose-stimulated insulin release by rat islets,[1,13,27-29] as well as the need for methodological precautions in this type of research.[30]

Verspohl and Ammon[28] reported that isolated islets from 24-month-old Wistar rats bind insulin more strongly and are more susceptible to feedback inhibition of glucose-stimulated insulin secretion in vitro than are isolated islets from three-month-old rats. Furthermore, the insulin-secreting capacity of 16.7 mmol (300 mg/100 mL) glucose was the same by incubated islets from three- and 24-month-old donor rats when insulin secreted into the incubation medium was bound to added anti-insulin serum. However, these investigators also failed to distinguish between islets of different sizes, as discussed above; ie, larger islets are more prevalent in aging rats,[13] secrete greater quantities of insulin than do smaller islets,[13] and contain greater numbers of beta cells per islet.[19] Whether the impairment of glucose-stimulated insulin release in vitro by a size-matched population of perifused or incubated small islets, as donor rats age, can be overcome by addition of insulin antibodies to the perifusion or incubation medium is not known.

MECHANISM OF ALTERED INSULIN RESPONSE

Change in Sensitivity to Glucose The mechanism that underlies the inability of perifused small islets from old rats to secrete insulin in vitro is not understood. However, the defect is probably explained in part by a decrease in islet sensitivity to glucose. Sartin et al[31] reported that the sensitivity of perifused small islets to glucose in vitro is reduced as fed donor Sprague-Dawley rats age from two to 24 months. The threshold glucose concentration for insulin response is increased from approximately 4 mmol (72 mg/100 mL) to more than 8 mmol (144 mg/100 mL). The maximal secretory response is reduced by 33%-50%. At less than maximal insulin response, the reduced sensitivity to glucose in vitro by small islets from fasted old rats was overcome by elevating the concentration of glucose in the perifusion medium to 27 mmol (500 mg/100 mL).

Elahi et al[32] used perfused intact pancreas to reveal a reduced insulin response to low but not to higher concentrations of glucose as donor Wistar rats aged from 12 to 23 months. Similar studies of the perfused pancreas by Reaven and coworkers[33,34] and by Curry and MacLachlan[35] conflict with one another, but included Sprague-Dawley and Fisher-344 rats only as old as 12 months. In view of the lesser impact of aging on insulin secretion by perifused large islets,[13] use of the perfused intact pancreas[32] may result in minimal age differences at high glucose concentrations. Furthermore, these data suggest that a defect in the regulation of insulin secretion during aging may be overcome in vivo by the capacity of old animals to compensate by expanding their pool of islet beta cells.[13,19]

Glucose usage by the islet beta cell may be an obligatory function in the glucose-stimulated secretion of insulin.[36] However, data on the impact of aging on islet glucose metabolism are conflicting. Reaven and Reaven[37] reported reduced rates of glucose oxidation to carbon dioxide at glucose concentrations of 16.7 mmol (300 mg/100 mL), using islet incubation systems from Sprague-Dawley rats aged two to only 12 months. In disagreement with Reaven and Reaven,[37] Burch et al[24] reported an increased rate of 10 mmol (180 mg/100 mL) glucose usage as rate of conversion of 3H-5-D-glucose to 3H-water, and also observed elevated levels of glucokinase, phosphofructokinase, and glucose-6-phosphate dehydrogenase with perifused average-sized islets from fed Wistar and Sprague-Dawley rats aged eight to 18 months. However, the islet populations isolated from older rats by these two sets of investigators are dissimilar. Using identical methodology to that of Burch et al for assessment of glucose usage,[24] Sartin et al[31] reported that a slight increase in glucose usage occurs in perifused small islets from fed two-month-old Sprague-Dawley rats as soon as the glucose concentration is elevated above 4 mmol (72 mg/100 mL) and is coincident with

biphasic insulin release. In perifused small islets from fed 24-month-old rats, glucose usage and insulin release do not increase until the glucose concentration is elevated to 8.35 mmol (150 mg/100 mL).[31] However, the islet population isolated from older rats by Sartin et al[31] differs from those utilized by Reaven and Reaven[37] and by Burch et al.[24] Ammon et al[38] reported no change in glucose usage nor in NADPH or NADH production during aging.

Draznin et al[39,40] concluded that the reduction in glucose-stimulated insulin release in vitro by isolated islets from aged rats results from impairment of glucose-induced margination of secretion vesicles at the plasma membrane, whereas lysis of marginated secretion granules remains intact as donors age. Insulin secretory response in vitro to a range of glucose concentrations was examined using an islet incubation system from two- and 18-month-old Fischer-344 rats. The process of secretion vesicle migration to the plasma membrane was assessed by measuring the glucose-stimulated recruitment of somatostatin receptors during exocytosis. Lysis of marginated granules was assessed by sequential perifusion of islets with varying concentrations of glucose; sodium isethionate, and inhibitor of granule lysis; and trifluoperazine, an inhibitor of granule margination.

Once again, values were not obtained in comparable islet populations at different ages. As already discussed above, the distribution of islet sizes and the insulin-secreting capability by islets of different sizes changes as donor rats age.[13] Draznin et al[39,40] express their data per unit of average islet diameter, as well as per number of islets, but fail to consider the possibility that insulin responsiveness differs when measured in islets of different sizes. Furthermore, this study excludes those islet populations that are most (40–80 μm, diameter) and least (350–400 μm, diameter) impaired as donor rats age.[13] Finally, it is impossible to distinguish between an age-related reduction in the magnitude of glucose-induced somatostatin binding in every islet and binding changes that characterize subpopulations of smaller and/or larger islets.

Impaired margination of secretion vesicles at the plasma membrane during aging[39,40] is a particularly attractive potential explanation for the delayed initiation of glucose-stimulated insulin secretion by small islets.[13] The secretory response is at least partially restored when small islets from old rats are incubated with glucose and antiserum to somatostatin.[41] However, Draznin et al[39,40] apparently were not aware of the relevant earlier literature.[13–18] In any event, it would be extremely useful to repeat these otherwise elegant studies in perifused small islets that are isolated from aging donors.

Differential Secretion of Stored and Newly Synthesized Insulin The time course of secretion of newly synthesized insulin molecules was examined by Kitahara and Adelman[13] using perifusion of small (40–80 μm, diameter) islets from two- and 24-month-old Sprague–Dawley rats with 16.7 mmol (300 mg/100 mL) glucose and 3H-labeled leucine, and binding of insulin secreted into the perifusate to anti-insulin serum previously bound to cyanogen bromide-activated Sepharose. The time course of the appearance of radioactive secretory product bound to insulin antibodies is identical for islets from each age group. Therefore, it was concluded that the secretion of newly synthesized insulin molecules by perifused small islets is not impaired as donor rats age. It was suggested further that during aging the secretion of newly synthesized insulin molecules is preferential to that of previously synthesized molecules, the mobilization of which for secretion apparently is impaired as donor rats age. This preferential secretion of newly to previously secreted insulin molecules had been reported previously for perfused islets from young rats by Sando and Grodsky.[42–46]

As part of broader investigations of effects of aging on insulin synthesis and secretion, Gold et al[47] and Wang et al[48] also observed a decrease in the secretion of newly made insulin in vitro by isolated islets from aging donor Sprague–Dawley and Fisher-344 rats, as well as preferential release of newly made insulin over preformed insulin at all ages studied, although earlier published reports are acknowledged incorrectly or not at all. One potentially serious difficulty in the interpretation of the former study[47] is the investigators' selection of dissimilar islet populations from rat donors of increasing age. It is not possible to distinguish between changes that are attributable to the expression of aging within islets of homogeneous size and/or to differential abilities of islets of different sizes, since the proportion of larger islets increases as donor rats age.[13–19] Furthermore, the utilized protocol intentionally selects from older donors an islet population that contains a greater proportion of larger islets than does the islet population selected from younger donors.

Interpretation of the study by Wang et al[48] is obfuscated in at least the following ways:

1) The isolated islets were reported to range in diameter from 60 to 190 μm, in contrast to reports by others who observed both somewhat smaller and much larger islets in various rat stocks including the Fisher-344.[13–19,39] It is not unlikely that differences in digestion technique may account for the limited distribution of islet sizes observed.[30]

2) The authors indicate that pilot studies revealed no differences in insulin secretion between small and large islets when results were normalized to amount of islet insulin. However, the observed approximately threefold range in islet size is probably also expressed as a corresponding threefold range in the amount of beta cells and insulin content per islet.[19] Therefore, insulin

secretion per islet probably increases correspondingly with islet size at any given age, as reported previously.[13]

3) The authors confirm earlier observations by numerous others that a greater proportion of larger islets is evident in older rats than is evident in younger rats.[13-19] Furthermore, insulin secretion is impaired to a much greater extent in small islets than in large islets during aging of donor rats.[13] Therefore, since a random sampling of islet sizes was utilized at all tested ages by Wang et al,[48] it is not possible to distinguish between changes that are attributable to the expression of donor rat aging within islets of homogeneous size and/or to differential abilities of islets of different sizes to secrete insulin.

4) Finally, the pulse-chase experiments with which secretion of newly synthesized and stored insulin was investigated were pursued over a four-hour period. The extent to which these data were influenced by the more prominent feedback inhibition which characterizes islets from older rats[28] was not investigated.

Change in Paracrine Control Several observations are consistent with the conclusion that changes in the regulation of glucose-stimulated insulin secretion by isolated islets from aging donors are not intrinsic to the islet beta cell, but instead may be secondary to alterations in islet paracrine control mechanisms that address the quality and/or quantity of endogenous glucagon and/or somatostatin that is available to beta cells within intact islets. For example, Molina et al[27] reported that any defect in islet glucose metabolism that interferes with insulin secretion must occur prior to the metabolism of trioses, since glyceraldehyde stimulates insulin secretion similarly by perifused small islets from fed two- and 13-month-old rats. However, their rationale was based on the questionable presumption, as discussed above, that islet glucose oxidation is diminished during aging. Furthermore, it remains to be seen whether insulin responsiveness to glyceraldehyde diminishes in rats older than 13 months. In any event, a more viable interpretation of the similar insulin response to glyceraldehyde during aging is that the insulin secretory response per se by the islet beta cell does not change as rats age.

Chaudhuri et al[41] reported that the impairment of glucose-stimulated secretion of insulin during aging is overcome partially when small islets from 24-month-old, fasted Sprague-Dawley rats are incubated with 16.7 mmol (300 mg/100 mL) glucose and rabbit serum containing antibodies to somatostatin. These results are similar to an earlier report of the restoration of impaired growth hormone secretion following infusion of old rats with antibodies to somatostatin.[49] However, interpretation of the islet data is complicated by a lack of information about the amount of endogenous somatostatin that interacts directly with beta cells within intact islets at different ages, as well as identification of the particular molecular species of rat pancreatic somatostatin[50] with which the utilized antiserum[41] interacts. An additional complicating factor is the increased size and adiposity that accompany aging in Sprague-Dawley rats under the defined environmental conditions.[41] However, hypersecretion of somatostatin was also reported previously for perifused and incubated, isolated islets from streptozotocin diabetic, as well as from obese and lean age-matched control Zucker rats aged five to 18 months.[51,52]

Thus far, changes in glucose-stimulated secretion of insulin during aging are not detectable when dispersed beta cells are isolated from islets of aging rat donors. Magal et al[53] described an insulin secretory response that is independent of rat donor age when partially purified beta cells from pancreatic islets of three- to 27-month-old Sprague-Dawley and Fisher-344 rats are incubated with a glucose concentration elevated from 2.6 mmol (47 mg/100 mL) to 16.7 mmol (300 mg/100 mL). Physiological integrity of the cell preparation was demonstrated by, among other things, sensitivity of glucose-stimulated insulin secretion to inhibition by somatostatin in an identical fashion at 12 to 27 months of donor rat age. On the other hand, a potentially serious difficulty with these data is the small magnitude of insulin response to glucose relative to that observed previously for intact islets both by the same laboratory[13,41] and by others.[19] However, the low responsiveness is also in agreement with the results of others who examined dispersed islet cells.[54-58] The apparent absence of diminished insulin responsiveness to glucose by isolated islet beta cells as donor rats age[53] also suggests that feedback inhibition of glucose-stimulated insulin secretion by insulin from isolated islets of aged rats[29] either requires the integrity of an intact islet architecture or is susceptible to damage by the methodology utilized for dispersion of islet cells.

Adenylate Cyclase-cAMP Lipson et al[59] suggested that the adenylate cyclase-cAMP system plays a role in the impairment of glucose-stimulated insulin release during aging. Basal levels of islet adenylate cyclase activity were reduced by 57% as donor Sprague-Dawley rats aged from three to 15 months, whereas phosphodiesterase and protein kinase activities were unchanged. However, basal levels of insulin secretion were identical in these two groups of islets. Further difficulties in the interpretation of these data include: the failure to measure enzyme activities in glucose-stimulated islets in which rates of insulin secretion diminish as donor rats age; the failure to distinguish between aging, growth, and obesity; and the failure to specify islet size.

CONCLUSIONS

Research on the regulation of insulin secretion by glucose during aging has not progressed very far. It seems clear that the intact pancreas maintains its ability to

secrete insulin rapidly in response to changes in blood glucose. However, the manner in which glucose stimulates insulin secretion apparently differs in young and old individuals. The predominant population of pancreatic islets of Langerhans progressively loses its ability to respond rapidly to a glucose challenge in vitro as donor rats age. At the same time, a morphologically distinct population of larger islets becomes more prevalent during aging, apparently accounting for the continued integrity of glucose-stimulated insulin response in vivo during aging. The mechanism by which the population of small islets loses its ability to secrete insulin rapidly in response to glucose during aging is unknown, although changes in glucose sensitivity and a role of paracrine regulation may be of importance. The nature of factors that regulate the distribution of heterogeneous islet populations at different ages is unknown. Whether the functional decline expressed by the population of small islets or the greater prevalence of the population of larger islets is of greater fundamental importance to the understanding of basic processes of biological aging is also unknown.

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